A METHOD FOR ESTIMATING HYDROPHILIC COLLOID CONTENT OF EXPRESSED PLANT TISSUE FLUIDS[1]

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(WITH ONE FIGURE)

In the preceding paper by GORTNER and HOFFMAN[2] it was pointed out that studies of the physico-chemical properties of plant saps which include only measurements of the osmotic pressure, electrical conductivity, and H-ion concentration, leave out of account the very important influence on physical properties exerted by sap colloids. By the introduction of the refractometer as a part of the field laboratory equipment, these workers have shown it possible to make rapid and accurate determinations of the moisture content of the plant saps. Utilizing the additional data thus made available, a simple method has been devised which appears to give a relative measure of the content of hydrophilic colloids.

The freezing point depression of the freshly expressed plant juice is first obtained. Then, having determined the total solids by the refractometric method, a quantity of sucrose just sufficient to make a molar solution in the total water present is added. The freezing point depression is again determined, and is usually found to have increased more than the theoretical amount.

The values for the excess depression recorded in this paper have been based on the assumption that sucrose forms the hexahydrate in solution. The evidence for this has recently been discussed by SCATCHARD,[3] who also contributed additional data. In preliminary experiments with pure sucrose dissolved in distilled water, freezing point depressions were obtained slightly in excess of those

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[2] GORTNER, R. A., and HOFFMAN, W. F., Determination of moisture content of expressed plant tissue fluids. BOT. GAZ. 74:308–313. 1922.

[3] SCATCHARD, G., The hydration of sucrose in water solution as calculated from vapor-pressure measurements. Jour. Amer. Chem. Soc. 43:2406–2418. 1921.

expected under this assumption, but it is preferred to use the theoretical value until further data on this point are available.

It is assumed that the magnitude of the excess depression is a measure of the quantity of water held in such a way as to be unavailable for the solution of the sugar. The values obtained may be calculated to percentage "bound" water. This represents the total water of hydration of all the substances in the sap, but has been found to correspond so regularly with the content of hydrophilic colloid as to indicate a close relationship. It seems probable that in most cases the water bound by substances other than colloids is of minor importance.

In table I are reported the data for a number of the samples of expressed juice included in table I of the preceding paper, and in addition for a series of gum acacia sols prepared by weighing out the necessary quantities of highly purified gum acacia and distilled water. The percentage of total solids, as read directly by the refractometer, is given in column 2. The values for viscosity, recorded in column 3, were determined by a viscosimeter of the Ostwald type, in a constant temperature bath at 25° C.; the figures are the number of seconds required for 3 cc. to flow through a capillary tube, through which the same quantity of distilled water flowed in 204 seconds. In column 4 is given Δ, the freezing point depression of the freshly expressed juice; in column 5 Δ_a, the freezing point depression after the addition of the sugar; in column 6 $\Delta_a - \Delta$, the actual additional depression due to the added sugar; in column 7 $\Delta_a - (\Delta + K_m)$, the amount by which the depression found on addition of the sugar is in excess of that expected on theoretical grounds. As previously noted, it has been assumed that sucrose forms sucrose hexahydrate in solution, and therefore K_m, the molecular constant for the depression of the freezing point, has been taken as 2.085° C. instead of the usual 1.86° C. The percentage "bound" water, given in the last column of the table, is conveniently calculated from the value for actual additional depression due to the added sugar ($\Delta_a - \Delta$). The calculations involved will be made clear in the following example, using the first item in the table.

1.86° = Δ due to 1 mole dissolved in 1000 gm. water, but 1 mole sucrose combines with 6 moles water. Thus 1 mole sucrose dis-

solved in 1000 gm. water = 1 mole sucrose hexahydrate dissolved in $1000-(18\times 6)$, or 892 gm. water, and 2.085° $(K_m)=\Delta$ due to 1 mole dissolved in 892 gm. water.

But in sample 13 the increase in Δ on addition of 1 mole sucrose $(\Delta_a-\Delta)$ was 2.339°, and $2.339^\circ=\Delta$ due to 1 mole dissolved in $\frac{1.86\times 1000}{2.339}=795$ gm. water; therefore the water bound per liter = $892-795=97$ gm., $=9.7$ per cent.

In laboratory practice it is most convenient to weigh out a fresh portion of the sap containing 10.0 gm. of water, add 3.422 gm. of sucrose, and redetermine the freezing point. The percentage of bound water is then given by the formula: $\frac{\text{Excess }\Delta}{\text{observed }\Delta-\text{sap }\Delta}\times 892$ or bound water $=\frac{\Delta_a-(\Delta+K_m)}{\Delta_a-\Delta}\times 892$.

Comparing sample 13 with sample 16, it will be seen that whereas the percentages of total solids vary widely, the percentages of bound water are not greatly different. A reference to the values for viscosity and Δ will indicate at once the marked difference in the physical properties of these two saps, due to the large content of colloidal material in sample 16, a fact which is strikingly reflected in the percentage of bound water. Again, dialysis showed that material similar to sample 17 contained approximately twice the quantity of colloids as was contained in material similar to sample 20, and these two samples differ widely in percentage bound water. These examples are cited to illustrate the application of the method. Discussion of the significance of the variations observed in the wheat varieties is reserved for a later paper on another subject.

The percentages of bound water obtained with gum acacia sols, as shown in the table, increase regularly with concentration. In fig. 1 the percentage concentration has been plotted against the percentage bound water. The logarithms of these values have also been plotted in the same figure. It will be seen that both of these graphs suggest an adsorption curve.

The advantages of sucrose as the solute in this method are as follows: (1) it is easily obtained in a high degree of purity; (2) the large molecular weight reduces errors in weighing; (3) its behavior

TABLE I

Materials used: leaves of	Total solids	Viscosity (water=204)	Δ	Δ_a	$\Delta_a-\Delta$	$\Delta_a-(\Delta+K_m)$	Bound water
	Per cent	seconds					Per cent
13. Triticum vulgare var. Turkey	13.5	360	1.273	3.612	2.339	0.254	9.7
15. Bryophyllum calycinum	5.9	235	0.474	2.555	2.081	−0.004	0.0
16. Cereus sp.	4.9	637	0.505	2.803	2.298	0.213	8.3
17. Triticum vulgare var. Buffum	17.8	419	1.719	4.158	2.439	0.354	13.0
18. Triticum vulgare var. Minhardi	8.5	285	1.147	3.824	2.137	0.052	2.2
19. Triticum vulgare var. Super	7.1	267	1.000	3.106	2.106	0.021	0.9
20. Triticum vulgare var. Super	9.7	292	1.085	3.279	2.194	0.109	4.4
Solutions of gum acacia							
1 per cent	1.0	309	0.005	2.147	2.142	0.057	2.37
3 per cent	3.0	487	0.013	2.186	2.171	0.086	3.53
5 per cent	5.0	684	0.025	2.221	2.196	0.111	4.50
7 per cent	7.0	932	0.034	2.254	2.220	0.135	5.42
10 per cent	10.0	1438	0.048	2.294	2.246	0.161	6.39

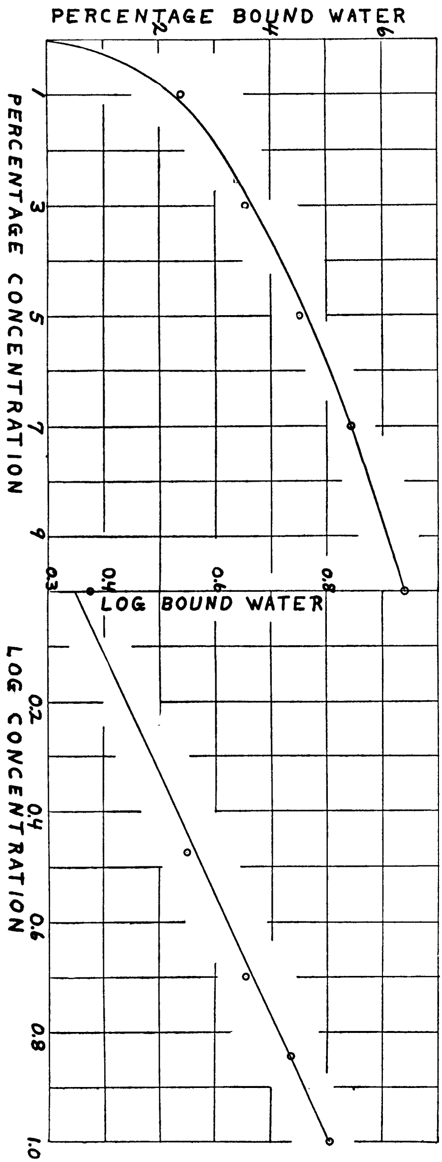

FIG. 1.—Relation between concentration of gum acacia sols and water bound by the colloid.

in solution is fairly well known; (4) its effect on the swelling of colloids is probably negligible. In objection it may be stated that plant saps probably contain invertase, but the plan has been followed of grinding the sucrose to a fine powder which dissolves rapidly with shaking, and the sap has been maintained continuously at low temperature. Under these conditions no increased depression of the freezing point which could be attributed to invertase action has been observed in a somewhat extended series of determinations on the same sample.

Conditions of equilibrium, and possible errors due to adsorption of the sugar by colloids, have not yet been investigated. The data already secured, however, appear to justify the proposal of the method for the estimation of the relative (not absolute) content of hydrophilic colloids in expressed plant tissue fluids. It seems probable that the method may be applied also to other biological fluids.

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